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Scale dependent responses of pine reproductive traits to experimental and natural precipitation gradients

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ABSTRACT

Drought considerably affects physiological and biochemical processes in trees and thus also reproduction. However, little is known about shifts in reproductive strategies in relation to changes in water availability.

We investigated cone and seed traits as well as needle and twig growth traits in pine trees along a natural precipitation gradient (290 mm–750 mm) and in a long-term irrigation experiment to understand reproductive responses to water availability. Combining multiple traits, we found that cone trait richness and divergence were positively correlated with water availability. This suggests drought-induced environmental filtering resulting in a reduced set of cone trait combinations. In contrast, seed trait diversity indices were negatively correlated with water availability, suggesting that pine trees tend to maximize seed trait dissimilarity in multiple dimensions to efficiently utilize scarce resources. Trade-offs between reproduction and vegetative growth were observed along the transect but not in the irrigation experiment, which may be a result of different drought severity between the two study regions.

Our results highlight the importance of considering a broad aridity gradient and different spatial-temporal scales when studying plant reproductive responses to precipitation change. Furthermore, the present study indicates that quantifying complementary components of trait diversity allows to interpret ecologically complex changes in trait distribution in multiple dimensions.

1. Introduction

Global precipitation patterns have exhibited apparent changes in the last century in both temporal and spatial scales (Stocker, 2014) and are expected to be further modified. Such changes will affect soil water availability and will also modify intensity and frequency of drought events (O'Brien et al., 2017a). Substantial evidence has revealed the impacts of changes in water availability on the physiological and biochemical processes in trees (Li et al., 2008a,b; Zhu et al., 2012; Li et al., 2013; Wagg et al., 2017). In this context, exploring how reproductive strategies of plants, particularly of long-living tree species, respond to changes in precipitation regime has become a hot topic of current ecological studies (Mutke et al., 2005; Way et al., 2010). Pine species are highly suitable to study this issue, given their wide geographical distribution, as well as their ability to adapt to different environmental conditions (Leslie et al., 2012).

Traits of single individuals determine their fitness (Violle et al., 2007; Siefert et al., 2015) by affecting responses to the environment and ecological interactions (Bolnick et al., 2011; Violle et al., 2012; Kraft et al., 2014). Phenotypic plasticity of functional traits, both in morphology and in physiology, is increasingly considered to be a crucial strategy for tree species to respond rapidly to altered environmental conditions (O'Brien et al., 2017b), and to be also of importance for evolution on longer time scales (Sultan, 2000). However, large parts of

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current research on trait plasticity are concentrated on vegetative organs, including shifts in needle area and longevity (Oleksyn et al., 2003), changes in branch traits (Adams et al., 2015), as well as adjustments in stem and fine root growth (Brunner et al., 2009; Eilmann et al., 2009; Wang et al., 2017). Little information is available on the intraspecific variability in reproductive traits and phenology of trees in relation to intra and inter-annual mean precipitation changes (Volis and Bohrer, 2013; Ayari and Khouja, 2014). This has so far limited a comprehensive understanding of tree eco-physiological responses to environmental changes such as altered water availability, particularly considering that reproduction is directly related to plant fitness, population dynamics and community structure (Thomas, 2011).

Most of the few studies dealing with intraspecific variability of reproductive traits focused on overall cone and seed yield per tree rather than on the variability of single cones and seeds within individual trees, which however can even exceed mean differences among trees (Mutke et al., 2005). In addition, functional traits generally do not vary independently but rather co-vary, and plants tend to adopt certain trait combinations (trait syndromes) to maximize their performance. It is thus crucial to consider variability of multiple traits simultaneously to quantify functional changes. To this end, trait diversity indices specifically designed for the use of individual-level trait data have been recently developed (Fontana et al., 2016). Trait richness (the amount of trait space covered by individuals of a population) and trait divergence (a measure of dispersion of individuals around the centroid of trait distribution) potentially reflect environmental filtering, because they are sensitive to losses of trait combinations represented in a certain population (Cornwell et al., 2006; Mouillot et al., 2013; Fontana et al., 2016). Trait evenness quantifies the regularity in trait distribution and thus is supposed to respond mainly to biotic interactions (e.g. competition), which are arguably more intense under limited resources, and to detect niche partitioning (Fontana et al., 2018).

Female cones and seeds are two structures with different reproductive functions in conifers, and cone and seed morphological traits are positively correlated with each other (Leslie et al., 2017). Moreover, seed size is closely linked with plant life history, which usually involves a trade-off between seed quality and quantity (Smith and Fretwell, 1974). This pattern has been widely confirmed at the interspecific level, where few but bigger seeds are commonly produced by species living in unfavorable environmental conditions (Leishman et al., 2000; Pluess et al., 2005). However, patterns of intraspecific seed variation within or across populations in response to altered water availability have so far received little attention and the same is true for cone traits.

Variation in plant reproduction in response to environmental changes involves a range of physiological processes, such as resource allocation among different functions or organs (Obeso, 2002; Thomas, 2011; Deyn, 2017). Under limited resources availability, increased resource investment in reproduction (i.e. reproductive allocation) may entail decreased allocation to vegetative tissues and functions, leading to trade-offs between reproduction and vegetative growth, as defined by the 'resource switching hypothesis' by Monks and Kelly (2006). However, this kind of correlation does not seem to be universal. For example, Despland and Houle (1997) found that changes in both reproduction and vegetative growth of *Pinus banksiana* in Subarctic Quebec simply matched variations in resource availability without any apparent trade-off, following the 'resource matching hypothesis' (Kelly, 1994). Which of these two hypotheses is applicable in a specific situation may depend on the spatial-temporal scale of analysis (Barringer et al., 2013; Vilà-Cabrera et al., 2014), type and range of stress gradients, ontogenetic stages (Genet et al., 2009) and hierarchical levels studied (Obeso, 2002), as well as underlying mechanisms (e.g. acclimation *vs*. adaptation). Despite recent efforts, our understanding on how resource allocation between reproductive and vegetative growth responds to changing water availability is still limited. Moreover, given the multi-year duration of the reproductive cycle of pine trees, it is necessary to study reproductive allocation across years (Pérez-Ramos et al., 2010).

To our knowledge, few studies have simultaneously combined natural transect and manipulation experiment to explore the functional response of trees to environmental changes. In this study, we investigated the reproductive (seeds and cones) and vegetative traits (needles and shoots) of pine trees in relation to water availability along a natural precipitation gradient and in a controlled long-term irrigation (i.e. precipitation manipulation) experiment. Our experimental design allows us to explore the pine reproductive strategies at different scales in the context of global precipitation changes. Such scales not only involve different time spans of pine trees exposed to drought, but also encompass a steep gradient of drought severity. We expected acclimation responses of reproductive traits (or phenotypic plasticity) of *Pinus sylvestris* L. to short-term changes in water availability in the irrigation experiment, and adaptive responses of *P. sylvestris* var. *mongolica* Litvinov to long-term persistence of the species along the natural precipitation gradient. By using *P. sylvestris* var. *mongolica* along the natural gradient and *P. sylvestris* in the irrigation experiment we assumed that observed patterns can be attributed to different underlying mechanisms rather than to the autoecology of trees (very similar in this case). We investigated the relationships between vegetative growth and reproduction, and calculated diversity indices of reproductive traits, to test the hypotheses that (I) trade-offs exist between seed size and number, as well as between reproduction and vegetative growth, (II) the strength of these trade-offs is inversely proportional to water availability, and (III) decreasing water availability causes lower trait richness and divergence (environmental filtering) but higher trait evenness (niche partitioning).

2. Material and methods

2.1. Transect study: study area, aridity gradient, sampling and measurements

The research was carried out in six *P. sylvestris* var. *mongolica* plantations with an age between 35–50 years along a 1200 km transect in Northeastern China (Fig. S1). In the six *P. sylvestris* var. *mongolica* plantations, all trees developed from seeds collected from the same natural *P. sylvestris* var. *mongolica* population in Honghuaerji (HHEJ) (47°35–48°36′N, 118°58–120°32′E), Hulunbeier/China, and were planted to prevent soil erosion (Zhu et al., 2003). They had a Sokal (1958)'s genetic simple matching (SM) coefficient of 86% (Fig. S2). Along the transect, mean annual temperature (MAT) ranges from 4.7 °C to 6.8 °C, with a tendency for slightly higher values in the West. The transect is characterized by a steep climate gradient with increasing aridity from East to West: mean annual precipitation (MAP) dramatically decreases from 748 mm (East) to 284 mm (West), while mean annual potential evapotranspiration (PET) sharply increases from 1206 mm (East) to 2300 mm (West). In addition, soils also show variability along the transect. The two easternmost sites (QD and BL) are located in the mountain areas of Jilin Province, where soils are silty and relatively nutrient-rich, with a thick organic layer. The other four sites (FJ, ZG, NM and WL) are distributed in the Keerqin sandlands, where soils are sandy, nutrient-poor, loosely structured and susceptible to wind erosion (Fig. S1, Table S1).

To describe the water availability of each site along the transect, we calculated the aridity index according to Delgado-Baquerizo et al. (2013):

$$Aridity \quad index = 1 - MAP/PET \tag{1}$$

Where *MAP* represents the mean annual precipitation, *PET* represents the potential evapotranspiration. From West to East along the transect, *aridity index* declines from 0.88 to 0.38 (Table S1), indicating a decrease of aridity, due to increasing water availability. The climate data used in this experiment was obtained from the closest meteorological stations

with a distance of < 7 km from each site. The calculation of *PET* in these meteorological stations are based on the Penman-Monteith equation (Koster and Suarez, 1992).

In early November 2015, for each of the six sites, four plots at least 500 m apart from each other were selected for sampling. Four healthy and co-dominant trees of similar size and distant at least 50 m from each other, were randomly selected in each plot. Therefore, 16 trees per site (96 in total) were sampled along the transect. Mean diameter at breast height (DBH) of these trees ranged from 15.5 to 31.4 cm (Table S1). One leading branch without any visible damage was sampled from the middle and sun-exposed part of each tree crown with a long-handled twig shear for vegetative growth measurements. In the field, the annual shoot growth of the last 15 years was determined by measuring the annual length of the main shoot leader per branch for each tree, going back to the year 2001. Pine shoots can be dated accurately by counting back the internodes or age classes of the branches from the tip, and needles distributed on the annual shoots belong to the corresponding age class. All needles of each age class were sampled for subsequent measurements, separately put in envelopes and immediately stored in cooling boxes. At least 25 cones in total (Table S1) were sampled from the same trees across 4 plots per site. All sampled cones ripened in the previous year (further termed '*i*-1') and have fully opened their scales at the sampling date in the year *i* (i.e. all seeds had already dispersed and thus we did not collect seeds in the transect study – see Table S1).

In the laboratory, needle length was measured using a vernier caliper. Subsequently, all needles and cones were firstly oven dried at 65 °C for 48 h to constant weight. Six parameters determined to assess the reproductive traits of individual cones: dry weight of each empty cone ($cone_{empty}W$), cone length (coneL), cone diameter across the broadest portion (coneD), cone shape as the ratio between length and diameter, cone volume (coneV) and number of scales per cone (N_{scale}/Co). Cone volume (ConeV) was calculated using the following equation:

$$coneV = \frac{1}{3}\pi\left(\frac{1}{2}coneD\right)^2 \quad coneL \tag{2}$$

Where coneD represents cone diameter, coneL represents cone length. N_{scale}/Co was counted and used to indicate the potential number of ovules in each cone, since each scale usually contains two ovules (Goubitz et al., 2002).

2.2. Long-term precipitation-manipulation experiment: stand, irrigation regime, sampling and measurements

The study site was located in the Pfynwald forest (46°18′N, 7°36′E, 615 m *a.s.l.*), located in the Inner-Alpine Rhone valley of the canton Valais, Switzerland. The area is dominated by *P. sylvestris* with an approximate average stand age of 100 years and a stand density of 730 stems ha^{-1} with occasional *Quercus pubescens* Willd. mixed-in. The climate is continental with a mean annual precipitation of 605 mm and a mean annual temperature of 10.1 °C (June–August: 15.8 °C) for the period of 1981–2010 (Timofeeva et al., 2017). The soil is a skeletal pararendzina, with low water holding capacity. More details about stand structure and soil properties is given by Herzog et al. (2014) and Hartmann et al. (2017). In order to give a more intuitive comparison of the drought severity between the two research regions, we also calculated the aridity index under the control conditions of irrigation experiments (aridity index = 0.13).

The irrigation experiment started in spring 2003. The study area, including 899 numbered and geo-referenced pine trees, was divided into eight plots of 25 × 40 m, separated by 5 m buffering strips. Trees in four randomly selected plots were irrigated (treatment termed 'irrigation' hereafter) throughout the vegetation period (May–October) during rainless nights using water from an adjacent water channel, fed by the nearby Rhone river. Trees in the other four plots were growing under naturally dry conditions ('control' hereafter). Therefore, each treatment includes four plots as replicates. Irrigation doubled the long-term mean annual rainfall at the site (~1400 mm). Detailed information about the experiment could be found in Dobbertin et al. (2010). In October 2013, the irrigation treatment was stopped on sub-plots of 10 × 25 m within each of the irrigated plots, to stimulate the ecosystem response and resilience to drier conditions ('irrigation stop' hereafter), whereas the remaining part of each irrigation plot continued to be irrigated. Irrigation has been demonstrated to have significant effects on pine needle and shoot growth (Dobbertin et al., 2010). In September 2016, 37 *P. sylvestris* trees (12, 15 and 10 trees in 'irrigation', 'irrigation stop' and 'control', respectively) were randomly selected for vegetative growth measurements. For each sampled tree, six branches were selected from the crown and their positions (upper and/or middle crown section) and vigor status (strong, intermediate, and/or weak) were visually evaluated and recorded. We used the same method as in the transect study to determine the annual shoot growth in the last 15 years for each tree. At the same time, we measured in the field the length of all needles attached on the branches. Cones were sampled between 13 and 16 February 2017. A total of 44 trees (19, 13 and 12 in 'irrigation', 'irrigation stop' and 'control', respectively) were selected randomly for cone sampling. All the cones from the whole crown of each tree were harvested. Theses cones have ripened at the sampling date (in the year *i*), but still not opened.

Back in the laboratory, collected cones were heated in an oven at 45 °C for 48 h to fully open the scales. Seeds were extracted from the opened cones and the filled seeds were separated from the empty ones by a blower. The empty cones were dried in the oven at 65 °C for about 48 h to constant dry weight. After that, cone and seed traits were measured. We firstly determined the total number of cones. Total dry weight of the empty cones and of the filled seeds was measured. A random sample of 324 seeds in total (132, 100 and 92 from 'irrigation', 'irrigation stop' and 'control', respectively) was selected for seed length and width measurements. In addition, three replicates of 100 seeds per tree were randomly selected and weighed to calculate mean seed weight. Additionally, a random sample of 148 cones was selected (62, 44 and 42 from 'irrigation', 'irrigation stop' and 'control', respectively) for cone trait measurements. To calculate the three additional cone traits compared to the transect study, we firstly calculated the number of filled seeds per tree by dividing the total weight of seeds per tree by the mean individual filled seed weight. Then $N_{filledseed}$/Co and $W_{filledseed}$/Co were calculated using the total number and total weight of filled seeds per tree divided by the number of empty cones per tree. $Cone_{filled}W$ was calculated as the sum of the mean individual empty cone weight and the total weight of filled seeds for each cone.

2.3. Measurement accuracy

For morphological measurements of cone and seed, all length measurements were performed with a Vernier caliper (accurate to 0.01 cm), whereas weights were determined with an electronic balance (accurate to 0.01 mg).

2.4. Reproductive trait diversity

Three indices, *i.e.* TOP ('trait onion peeling'), TED ('trait even distribution') (Fontana et al., 2016) and FDis ('functional dispersion') (Lázaro and Traveset, 2009), were calculated using the above-mentioned reproductive traits to describe the three main components of trait diversity (Mason et al., 2005): richness, evenness and divergence, respectively. This set of indices has been proven to accurately quantify trait diversity when intraspecific variability in multidimensional trait space needs to be considered (Fontana et al., 2016), as it is the case in the present study. To avoid excessive autocorrelation between traits, we calculated Pearson's *r* across all samples of the transect study (cone traits) and the irrigation experiment (seed and cone traits), separately. Considering 0.7 as the maximum acceptable absolute pair-wise

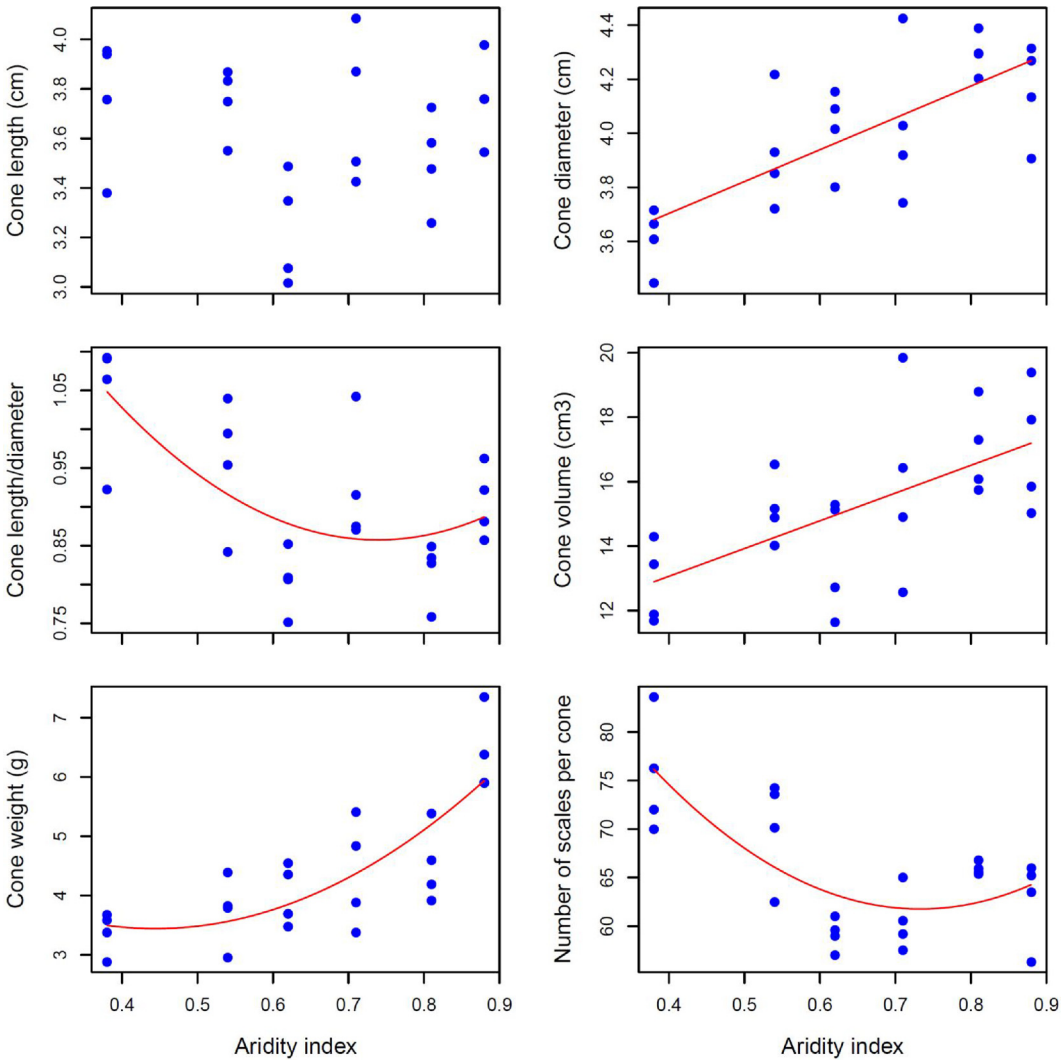

Fig. 1. Relationships between traits of single cones and aridity for *P. sylvestris* var. *mongolica* along an aridity transect in Northeastern China. Solid lines indicate significant regressions ($p < 0.05$).

correlation (Dormann et al., 2013), we selected the most representative cone and seed traits and standardized them (mean = 0, SD = 1) prior to indices calculation, to ensure that variation in each trait had the same importance. The traits selected to calculate trait diversity indices and their ecological relevance are listed in Table S5. Since sites (transect study) and treatments (irrigation experiment) had samples of different size, we performed a bootstrap procedure to randomly select 10 or 30 reproductive organs (in the transect and irrigation study, respectively) from each site/treatment 999 times. We then calculated trait diversity indices for each of these subsamples, considering cone and seed traits as multidimensional space axes.

2.5. Reproductive effort and vegetative growth rate

Both, in the transect study and irrigation experiment, the measured reproductive traits were used to calculate the reproductive effort (RE) of pine trees, while needle length and annual shoot growth were used as measures of vegetative growth rate (VR). The development of female cones in *P. sylvestris* trees takes two years, i.e. from cone initiation and growth in the year *i*-1 to seed ripening and dispersion in the year *i*. To detect the vegetative growth-reproduction trade-off prior reproductive formation, we measured and analyzed the vegetative growth in year *i*-2. To investigate how VR involved in the reproductive cycle of pine trees affects reproductive effort, we therefore measured VR in the transect study using needle and shoot length data of the last three years, i.e. from 2013 (*i*-2) to 2015 (*i*). There was a special case where we only used needle length data of the last two years to measure VR, due to the lack of needles of the year *i*-2 at the easternmost site (QD). However, we only considered needle and shoot length data of the year 2015 (*i*-2) and 2016 (*i*-1) to measure VR in the irrigation experiment, because needle and shoot buds of the year 2017 did not appear yet at the sampling time. Additionally, to eliminate the effects of tree size on annual shoot growth, the latter was expressed as the percentage increase in shoot length, i.e. shoot relative growth rate (*RGRs*), which was calculated according to Eq. (3):

$$\mathrm{RGRs} = SLi/MSL(\%) \quad (3)$$

where *SLi* represents the annual shoot length of the year *i* (2013, 2014 and 2015 for the transect study, 2015 and 2016 for the irrigation experiment), while *MSL* is the mean length of the shoots grown between 2001 and 2012 for the transect study, and between 2003 and 2014 for the irrigation experiment (both for a 12-year period). We used a ratio of RE to VR ('RE/VR' ratio hereafter) to express reproductive allocation, as well as to understand how it changed in response to water availability and year of vegetative growth. For this ratio, VR was still assessed using needle length and RGRs as mentioned above, while RE was based on

two traits (i.e. coneV and $cone_{empty}W$) in the transect study and on six traits (i.e. coneV, $cone_{empty}W$, seedW, $N_{filledseed}/Co$, $W_{filledseed}/Co$ and $cone_{filled}W$) from the irrigation experiment.

2.6. Data analysis

Linear regression analysis was performed in the transect study to assess the effects of water availability (W) on single reproductive traits and trait diversity indices, where we applied ANOVA to test if the inclusion of a quadratic term (allowing for curvilinear relationships) improved the model fit, as well as the effects of water availability, year and their interactions on reproductive allocation. ANOVA analysis was performed in the irrigation experiment to assess the effects of water availability on single reproductive traits and on TD indices and the effects of water availability, year and their interactions on reproductive allocation. Pearson's correlation analysis was performed to test the relationships between seed and cone traits in the irrigation experiment and between RE and VR in the transect study (combining all sampling sites) and in the irrigation experiment (combining all treatments and also for each treatment separately). All statistical analyses were performed with R version 3.1.3 (R Development Core Team 2015), and statistical significance was set at $p < 0.05$.

3. Results

3.1. Relationships between reproductive traits and water availability

3.1.1. Transect study

ConeD and coneV were positively related to aridity index (straight line) (both $p < 0.001$, $R^2 = 0.53$ and 0.38, respectively; Fig. 1), while cone shape (cone length/diameter), $cone_{empty}W$ and N_{scale}/Co showed a quadratic relationship (convex parabolic function) with AI (all $p < 0.01$, $R^2 = 0.38$, 0.62 and 0.49, respectively; Fig. 1). The correlation between aridity index and coneL was not significant ($p = 0.75$ and 0.23 with and without quadratic term, respectively).

3.1.2. Irrigation experiment

All reproductive traits significantly varied among treatments, with the exception of $W_{filledseed}/Co$ ($p = 0.37$), seed length ($p = 0.22$) and seed width ($p = 0.33$; Fig. 2). Cones collected from the 'irrigation' and 'irrigation stop' treatments had significantly higher values of coneL, $cone_{empty}W$, N_{scale}/Co, $N_{filledseed}/Co$ and $cone_{filled}W$ (all $p < 0.05$) than those collected from the 'control'. However, values of coneD, coneV and seed weight were significantly higher for 'irrigation' than for 'irrigation stop' and 'control' (all $p < 0.05$). Cone L/D showed the highest value in the 'irrigation stop' ($p < 0.01$; Fig. 2).

3.2. Trait diversity indices

3.2.1. Transect study

In the transect study, TOP and FDis showed a very similar quadratic relationship (convex parabolic function) with aridity index (both $p < 0.001$, $R^2 = 0.44$ and 0.30, respectively; Fig. 3). Both indices reached the highest values at the two wettest (and easternmost) sites, i.e. QD and BL. The following site towards West (FJ, with intermediate aridity index values) marked an abrupt and dramatic decrease (minimum value of both indices along the whole transect). Variation in TED along the transect followed a pattern similar to TOP and FDis (convex-shaped curve; Fig. 3), but although site FJ also represented the absolute minimum, the two wettest sites QD and BL in the easternmost site of the gradient did not show maximum values for TED. In fact, TED showed the tendency to gradually increase with increasing aridity index, but both with and without quadratic term, the explained variance was low (both $p < 0.001$, $R^2 = 0.09$ and 0.05, respectively).

3.2.2. Irrigation experiment

For both individual seed and cone measurements, all trait diversity indices significantly varied among treatments (Fig. 3). Again, patterns of TOP and FDis were particularly similar. In the case of seeds, 'control' always showed the highest values; for TOP and FDis, the difference to 'irrigation' and 'irrigation stop' treatments were very pronounced, whereas TED showed a gradual increase from 'irrigation' to 'irrigation stop' and 'control'. For cones, TOP and FDis reached the highest values in 'irrigation stop' and the lowest in 'control'. On the contrary, TED showed a gradual decrease from 'irrigation' to 'irrigation stop' and 'control' (Fig. 3).

3.3. Relationships between reproduction and vegetative growth

3.3.1. Transect study

We observed that almost all cone reproductive traits were negatively correlated with shoot relative growth rate in the year *i*-2 and *i*-1. However, these negative correlations almost disappeared in the year *i*. Particularly, $cone_{empty}W$ was significantly correlated with shoot relative growth rate in this year ($p < 0.001$, $R = 0.47$; Table 1). Similarly, almost all cone traits were negatively related to needle length in the year *i*-2, *i*-1 and *i* (Table 1). Reproductive allocation ('RE/VR' ratio) was influenced negatively by water availability. Only in few cases, year had an influence on 'RE/VR' ratio. No interaction effects between water availability and year were observed (Fig. 4)

3.3.2. Irrigation experiment

Almost all reproductive traits (both at the seed and cone level) were positively correlated with shoot relative growth rate and needle length in the years *i*-2 and *i*-1 (Table 1). The same results were found when correlations between RE and VR were assessed for each treatment (Table S3). Only cone length/diameter showed a negative correlation with shoot relative growth rate and needle length in the year *i*-2 (both $p < 0.01$, $R = 0.50$ and 0.37, respectively; Table 1). Water availability had a positive influence on 'RE/VR' ratio. Only in few cases, year had an influence on the reproductive allocation. No interaction effects between water and year were observed in this experiment (Figs. 5 and S3).

3.4. Relationships between cone and seed traits

In the irrigation experiment (during which we measured both, seed and cone reproductive traits), $N_{filledseed}/Co$ and $W_{filledseed}/Co$ were correlated with all cone traits ($p < 0.001$), except for cone shape (cone length/diameter; Table 2). Similarly, seed length and seed weight were correlated with coneL, coneD, coneV and $cone_{empty}W$ (all $p < 0.05$), whereas seed width only showed a correlation with $cone_{empty}W$ ($p < 0.05$, $R = 0.31$; Table 2).

4. Discussion

We found that the reproductive trait responses depended on how long trees were exposed to dry growth conditions. This is illustrated by comparing the different treatments in the irrigation experiment. The lowest values of cone trait richness and divergence were found in the control plots (mean TOP = 40.67, mean FDis = 1.36; Fig. 3), where trees were exposed to dry condition (long-term mean annual precipitation of about 600 mm in the last > 100 years). In contrast, the highest values for both indices were found in the 'irrigation stop' plots (mean TOP = 64.24, mean FDis = 1.72; Fig. 3). In this latter treatment, trees were irrigated for ten years (2003–2013) and then irrigation was stopped in 2014. This 'irrigation stop' treatment not only lead to higher trait richness and divergence compared to the control, but also exceeded the 'irrigation' treatment. Such patterns might not be expected as the water availability conditions of the 'irrigation stop' treatment converged within two years back to control levels and thus were intermediate between 'irrigation' and 'control'. Therefore, we rather

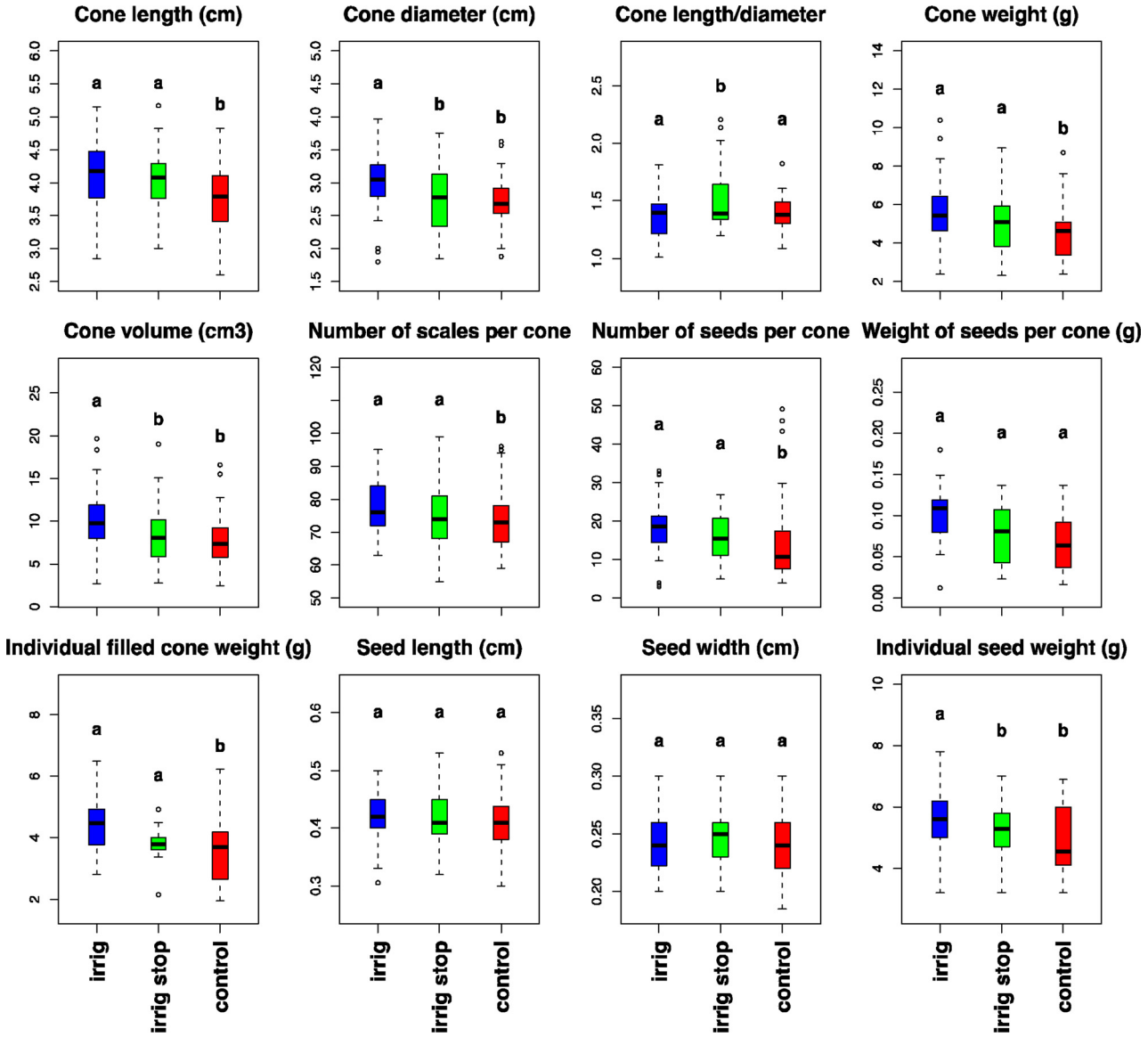

Fig. 2. Differences in traits of single cone and seed of *P. sylvestris* in an irrigation experiment in Pfynwald, Switzerland. Different letters indicate significant differences among treatments ($p < 0.05$). Abbreviations: 'irrig' represents continuous irrigation since 2003 and 'irrig stop' stopped irrigation since 2014, whereas 'control' is natural precipitation.

expected intermediate values of cone trait richness and divergence. The observed increase in trait richness and divergence was probably due to short-term acclimation, which started to shift trait distribution towards the optimum to survive under dry conditions. This rapid adjustment probably occurred before other trait combinations were filtered out by reduced water availability. As a consequence, our snapshot has probably caught a situation where many phenotypes were still represented in the population, and thus increased trait richness and divergence. However, we found that cone trait richness and divergence in the transect study followed a convex parabola (Fig. 3) rather than a monotonic decrease with increasing drought. This suggests that other abiotic factors rather than water availability determine changes in cone traits along the transect. For example, the silty soil with high organic content might also explain why the two easternmost sites showed very similar values for trait richness and divergence, while when moving further West, we observed an abrupt decrease in these two trait diversity indices (Fig. 3).

Simultaneous reduction in cone trait richness and divergence with decreasing water availability in both the transect study and the irrigation experiment (with exception of the 'irrigation stop' plots and the right side of the convex parabola fitted along the transect, as mentioned above) indicated that drought as an environmental filter determined changes in cone traits. In detail, the inviable cone phenotypes would be filtered out by severe drought stress, and only optimal phenotypes that maximized reproductive performance could survive. In contrast, the patterns of seed traits in the irrigation experiment seemed to contradict environmental filtering, because the maximum values of seed trait richness and divergence were found in the control treatments (Fig. 3). Apparently, the growth condition with higher water availability in irrigated plots allowed trees to converge towards the production of bigger seeds (Fig. 2), resulting in lower trait richness and divergence as compared to control trees. Moreover, trees under dry conditions in the control plots may diversify their seeds (i.e. higher seed trait evenness) to increase their chance of survival. The gradual increase in regularity of seed trait distribution with increasing drought severity (control *vs.* irrigation) and duration (control *vs.* irrigation stop) in the irrigation experiment suggests niche partitioning (Fontana et al., 2018). Since seeds are directly related to seedling development, we can assume that the increase in trait evenness, which maximizes trait dissimilarity in multiple dimensions for a given trait range, can be interpreted as a

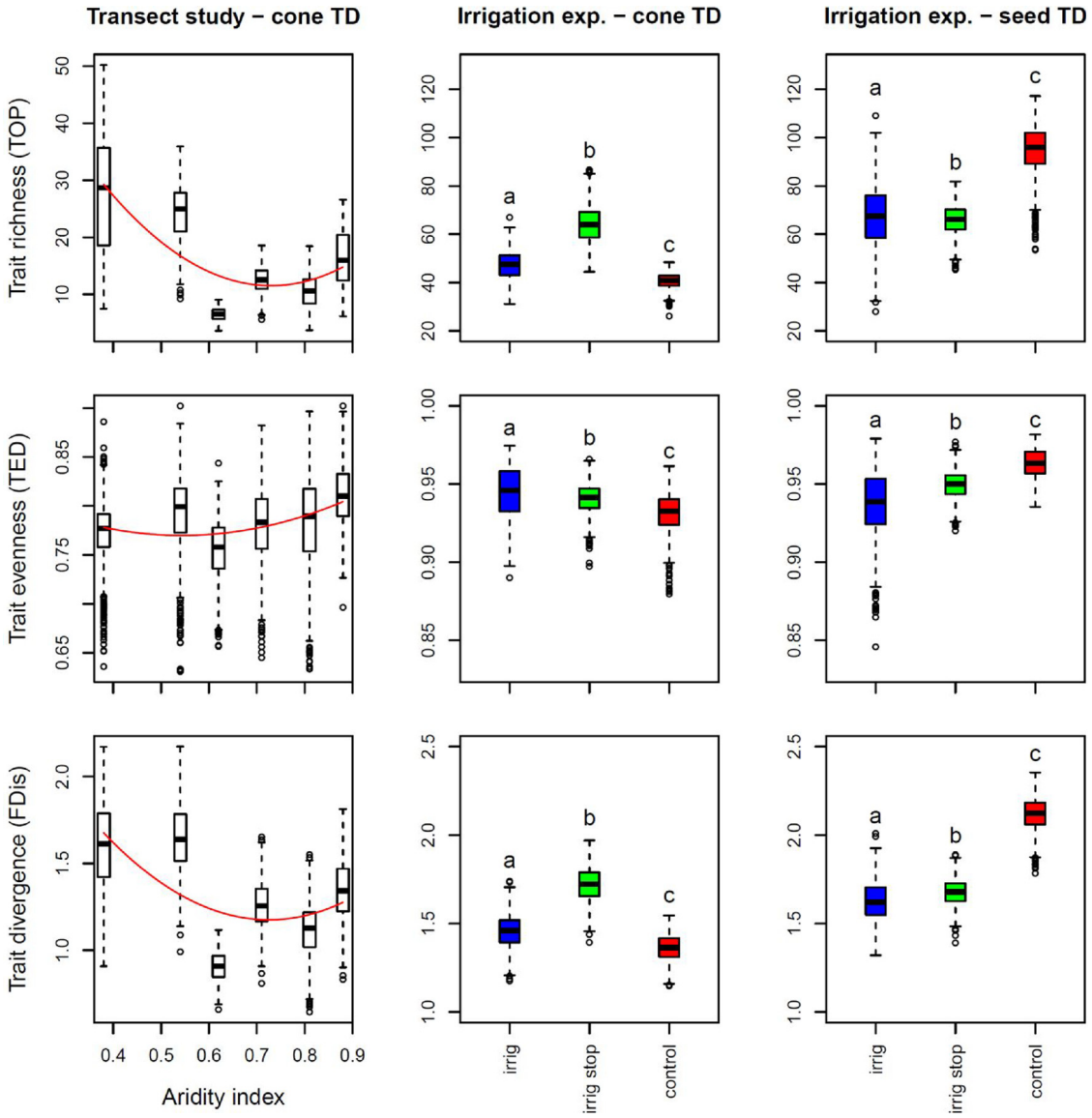

Fig. 3. Variation in trait diversity (TD) indices along an aridity transect and in an irrigation experiment. Different letters indicate significant differences among treatments ($p < 0.05$) in the irrigation experiment. *Abbreviations:* 'irrig' represents continuous irrigation since 2003 and 'irrig stop' stopped irrigation since 2014, whereas 'control' is natural precipitation.

strategy to minimize competition among seeds of the same individual under harsh conditions (Swenson and Enquist, 2009; Kraft and Ackerly, 2010). For example, as seed size is related to depth of the root system in seedlings (Bladé and Vallejo, 2008), higher trait evenness in seeds might be reflected in niche partitioning across soil layers, which would allow a more efficient resource use under drought.

Unlike for seeds, trait evenness for cones showed the minimum value in the control plots of the irrigation experiment (Fig. 3). This is consistent with the patterns of trait richness and divergence for cones, which suggests the convergence of the cone phenotype towards an optimum under dry conditions, and might be explained by the fact that cones are probably involved in niche partitioning less directly than seeds. On the contrary, cone trait evenness tended to increase with increasing aridity along the natural precipitation gradient (Fig. 3). However, in this case we observed a very low effect size (Table S2).

Given that our combined studies covered a wide range of water availability, *P. sylvestris* var. *mongolica* in China faced more severe drought stress (280–750 mm rainfall per year) than *P. sylvestris* in Switzerland (600 mm (control) – 1400 mm (irrigation) per year). Consequently, trade-offs between vegetative growth and reproduction, and between single cone size and scale number (i.e. seed number) per cone, were found along the transect only (Table 1 and Fig. 4), which may be explained by the resource switching hypothesis (Monks and Kelly, 2006). Similar results were also reported by Vilà-Cabrera et al. (2014), who suggested that reproduction of *P. sylvestris* in Spain would also occur at the expense of vegetative growth under dry conditions. Furthermore, the negative correlations between reproduction and shoot growth rate in the transect study disappeared in the year *i*, when the cones were matured (Table 1). This finding suggests that the competition for resources between shoot growth and reproduction occurred in previous years (*i*-1, *i*-2 year), when the reproductive organ formed, emerged, and developed. Similar results were also reported by Nussbaumer et al. (2016), who have confirmed that pine reproduction was also restricted by the growth during the preceding period.

In contrast, reproduction of *P. sylvestris* in the irrigation experiment followed the resource matching hypothesis by Kelly (1994). As the strength of trade-offs between reproduction and growth highly depends on the level of environmental stress (Obeso, 2002). The absence of trade-offs between growth and reproduction for *P. sylvestris* in the irrigation experiment might thus be due to the fact that the yearly

Table 1
Pearson correlations (r) between vegetative growth and reproduction along a precipitation gradient and in a long-term irrigation experiment.

	Shoot relative growth rate*			Needle length*		
	i – 2	*i* – 1	*i*	*i* – 2	*i* – 1	*i*
Transect study						
Cone length	−0.59**	−0.03	0.24	−0.50*	−0.25	−0.57**
Cone diameter	0.13	−0.33	0.05	0.11	−0.22	0.09
Cone length/diameter	−0.54**	0.10	0.15	−0.10	0.23	−0.38
Cone volume	−0.07	−0.40*	0.09	0.02	−0.23	−0.18
$Cone_{empty}W$	−0.30	−0.57**	0.47*	−0.61**	−0.42*	0.01
Number of scales per cone	−0.47*	−0.18	−0.02	−0.06	0.11	−0.08
Irrigation experiment						
Cone length	−0.05	0.16	Shoots in this year were not matured when sampling	−0.08	0.13	Needles in this year were not matured when sampling
Cone diameter	0.39*	0.07		0.24	0.18	
Cone length / diameter	−0.50**	0.03		−0.37*	−0.14	
Cone volume	0.31	0.08		0.13	0.13	
$Cone_{empty}W$	0.21	0.33		0.19	0.01	
Number of scales per cone	0.45*	0.14		0.43	0.33	
Number of seeds per cone	0.07	0.04		0.25	0.08	
Weight of seeds per cone	0.08	−0.08		0.13	0.19	
$Cone_{filled}W$	0.10	0.27		0.17	−0.05	
Seed length	0.26	0.09		0.15	0.10	
Seed width	0.25	0.36*		0.27	0.19	
Seed weight	0.27	0.21		0.38*	0.35*	

Abbreviations: i = current year; i – 1 = previous year; i – 2 = year before previous year. $Cone_{empty}W$ and $cone_{filled}W$ represent the weight of empty and filled cones, respectively. *, **, and *** indicate the significance level at $p < 0.05$, 0.01 and 0.001, respectively.

precipitation (> 600 mm per year) and, probably also other resources, were less limiting compared to the sites in the transect study. For example, the aridity index calculated for the control plots in Pfynwald was 0.13, that is much lower than the moistest site (aridity index = 0.38) of the transect study (Table S1). No trade-offs observed may also be possible that both reproduction and growth of trees were constrained to the same extent by drought stress, leading to the positive correlations even under extreme conditions (Despland and Houle, 1997). Additionally, tree reproductive costs may not only be reflected in reduction of current growth, but also in future reproduction, i.e., demographic costs (Santos-del-Blanco and Climent, 2014). According to the resource matching principle, trade-offs often occur between adjacent organs. For this reason, our study used needle length and single cone morphology traits (size and mass) to indicate growth and reproductive effort, respectively. However, we are aware that there are still some limitations in using only needle and shoot growth as measure of tree vegetative growth. In detail, the reproductive allometry may also occur based on other currencies. For example, Dombroskie et al. (2016) have recently demonstrated that the tree reproductive investment may involve the significant relationships between leafing and fruiting intensities (between total leaves number and potential fecundity). Moreover, the trade-offs between reproduction and growth could also be detected at physiological level, such as the carbon allocation of trees (Sala et al., 2012). All the above aspects indicated that further research should focus on many other currencies related to tree growth and reproductive efforts. Although previous studies have also argued that relative investment in tree reproduction and growth is closely related to ontogenetic stages (Genet et al., 2009), it may not be the reason for the differences in correlations between reproduction and growth in our two experimental regions. This is because all *P. sylvestris* trees analyzed in our study have been at the reproductive mature stage. Additionally, we also explored how the relationship between reproduction and vegetative growth was affected by variation in drought severity. The resource investment in reproduction was promoted by drought along the transect (Fig. 4), but by increased water availability in the irrigation experiment (Figs. 5 and S3), further confirming that reproductive allocation of pine trees depended on the degree of drought stress.

The trade-off between single cone size and scale number (i.e. seed number) per cone agreed with the results of reproduction-growth trade-off discussed above. The *P. sylvestris* in the irrigation experiment did not show any water-induced trade-offs due to relative higher water availability of > 600 mm per year for the driest plots, which is consistent with studies on *Pinus halepensis* Mill. along a growing season precipitation gradient of 400–600 mm per year (Ayari et al., 2011). Although the sampling time in November along the transect did not allow to collect seeds, the widely accepted positive relationships between individual cone size and individual seed size as well as between numbers of scales and numbers of seeds per cone (Leslie et al., 2014) can be used to deduce the trade-off between seed size and seed number along the transect. The fact that trees tend to produce fewer but bigger offspring under dry conditions has been reported widely (Moles and Westoby, 2006; Loewe-Muñoz et al., 2016). The advantages of large seeds in dry environments include greater reserves to promote germination rate and speed as well as early growth rate (Gómez and Husband, 2004; Cendán et al., 2013). Large seeds produce big seedlings with extensive root systems, which are beneficial to reach and absorb water and other resources from deep soils in harsh environmental conditions such as limited water availability (Bladé and Vallejo, 2008).

The cone length/diameter ratio decreased with drought in the transect study (Fig. 1), indicating that cones at dry sites tended to produce relatively bigger woody scales at the cone base, as cone scales generally grow bigger and thicker from cone tip to base (McGinley et al., 1990). This structural feature has ecological implications and is beneficial for seed pre-dispersal protection, since large scales near the base of the cone increase the time needed for predators to open cones as well as to remove them from the branch (McGinley et al., 1990). This protection mechanism appears to be much more important at drier sites or in harsh environments, where plants or trees produce fewer but bigger seeds for their natural regeneration.

5. Conclusions

We observed inconsistent reproductive strategies for *P. sylvestris* trees grown along a natural precipitation gradient and in a long-term precipitation manipulation experiment. Our results showed that trade-offs only existed in the transect study, mainly due to higher severity of drought stress, which is partially in line with our hypothesis I. The strength of these trade-offs was inversely proportional to water

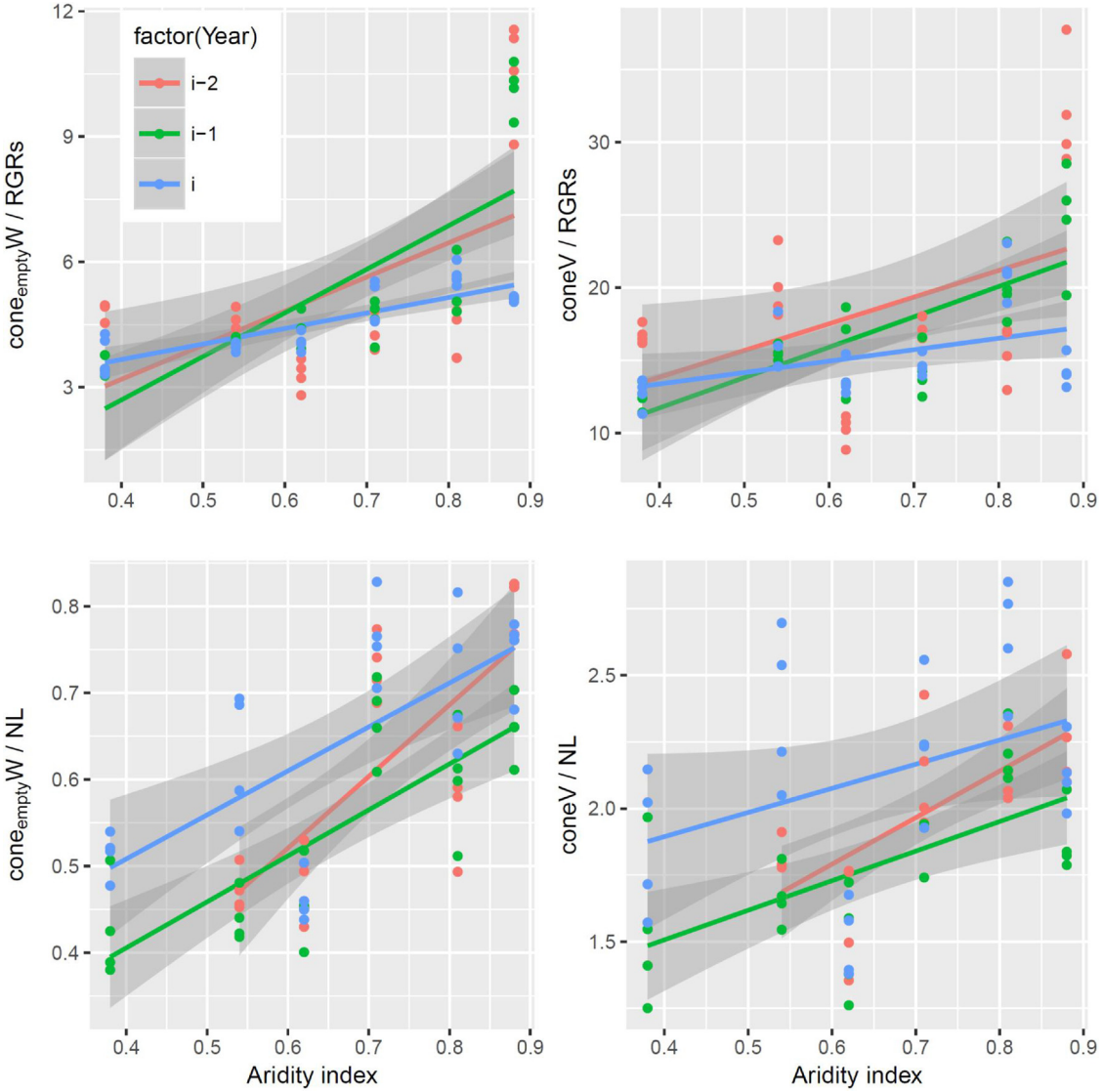

Fig. 4. Linear regression analysis to assess the effects of aridity, year, and their interaction on reproductive allocation along the precipitation gradient. Grey bands represent 95% confidence intervals. *Abbreviations:* cone$_{empty}$W, coneV represent single empty cone weight (without seeds) and cone volume, respectively; RGRs and NL represent shoot relative growth rate and needle length, respectively. *i = current year; i – 1 = previous year; i – 2 = year before previous year.*

availability, agreeing with our hypothesis II. The patterns of cone and seed traits diversity indicated different underlying mechanisms. Specifically, decreased cone trait diversity mainly suggested environmental filtering by drought stress. In contrast, seed trait diversity increased with drought, indicating that pine trees tended to maximize seed trait dissimilarity in multiple dimensions to efficiently utilize scarce resource under stressed conditions. Our study suggests that the reproductive responses of pine trees to drought depend on both drought intensity and exposure time, which highlights the importance of considering a broad gradient of drought stress and different spatial-temporal scales when studying plant reproductive responses to precipitation change, in order to cover mechanisms ranging from short-term phenotypic plasticity and acclimation to long-term adaptation. Although we are aware that our study design did not allow to completely disentangle the effects of drought intensity and exposure time through combining a natural rainfall transect and a manipulated irrigation experiment, analyzing trait variation with multidimensional trait diversity metrics allowed us to gain additional insights into the response patterns to aridity. Another limitation of the present study is our short investigation time that does not allow us to cover the occurrence of masting year, which will limit to understand the effects of masting on the trade-offs between vegetative growth and reproduction. Future work in this field should investigate additional traits potentially relevant for growth-reproduction trade-offs, using long-term reproductive monitoring data. Our results contribute to a better understanding and a more precise prediction of tree regeneration potential in a changing world.

Declarations of interest

None.

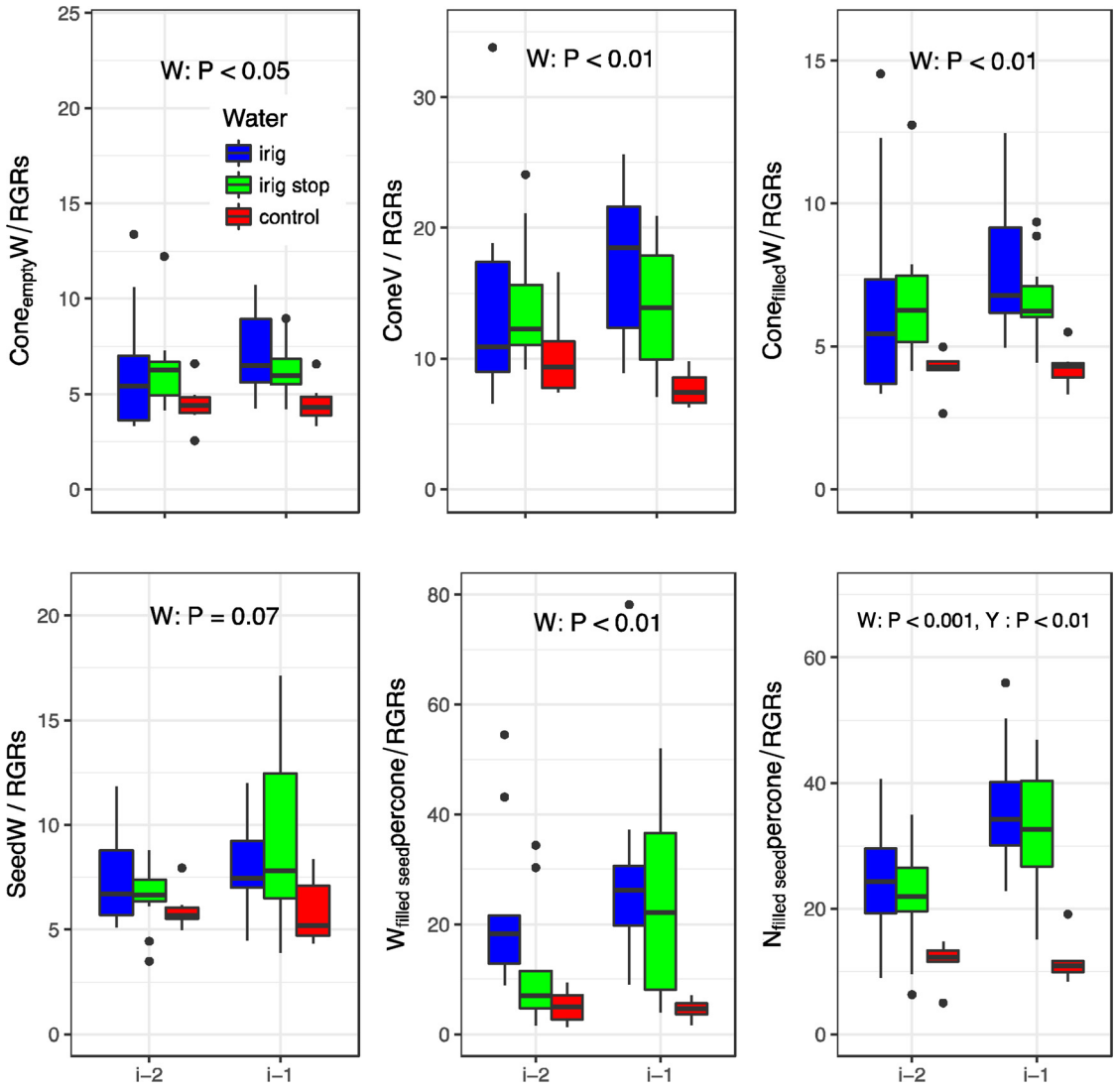

Fig. 5. Effects of water availability (W), Year (Y), and their interaction (W × Y) on reproductive allocation, using RGRs as a proxy for vegetative growth in the irrigation experiment. *Abbreviations:* Cone$_{empty}$W, coneV, cone$_{filled}$W, Seed weight, W$_{filledseed}$ per cone and N$_{filledseed}$ per cone represent single empty cone weight, cone volume, single filled cone weight, single seed weight, total weight of filled seeds, and total number of filled seeds per cone, respectively; RGRs represents shoot relative growth rate. *i = current year; i - 1 = previous year; i - 2 = year before previous year.*

Declaration of contributions

Peng He and Simone Fontana processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. Simone Fontana performed the trait diversity calculation. Peng He and Xin Sui carried out the sampling work and samples measurements. Maihe Li and Arthur Gessler devised the study, the main conceptual ideas, and proof outline. Maihe Li and Yong Jiang supervised the project. Marcus Schaub, Andreas Rigling and Yong Jiang aided in interpreting the results and worked on the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Table 2
Pearson correlations (r) between traits of a single cone and a single seed of *Pinus sylvestris* L. in a long-term irrigation experiment.

	Cone length	Cone diameter	Cone volume	Cone$_{empty}$W	N_{scale}/Co	Cone length/diameter
Seed length	0.49***	0.51***	0.53***	0.46**	0.05	−0.13
Seed width	0.27	0.27	0.27	0.31*	−0.04	−0.04
Seed weight	0.56**	0.31*	0.37*	0.61***	0.18	0.14
$N_{filledseed}$/Co	0.51***	0.57***	0.62***	0.56***	0.53***	−0.18
$W_{filledseed}$/Co	0.59***	0.56***	0.64***	0.67***	0.52***	−0.11

Abbreviations: cone$_{empty}$W and N_{scale}/Co represent weight of empty cones and number of scales per cone, respectively. *, **, and *** indicate the significance level at $p < 0.05$, 0.01 and 0.001, respectively.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2018.08.028.

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